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Phytochemical profiling of volatile and bioactive compounds in yellow mustard (*Sinapis alba*) and oriental mustard (*Brassica juncea*) seed flour and bran

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ABSTRACT

The objective was to study the phytochemical profile of yellow (*Sinapis alba*) and oriental mustard (*Brassica juncea*) flour and bran fractions. The HS-SPME-GC/MS technique was used to determine the volatile profile. Up to 53 volatiles, including isothiocyanates, alkanes, alcohols, ketones and esters were identified in the mustard powders. In addition, the free and bound bioactive fraction was studied and the determination was performed by UHPLC-MS/MS. A total of 26 compounds (phenolic acids, flavonoids and glucosinolates) were identified in the mustard samples. *Sinapis alba*, including the bran fraction, was richer in *p*-hydroxybenzoic, ferulic and *p*-coumaric acids compared to *Brassica juncea*. In addition, the volatile profile in yellow mustard seeds, as analysed by HS-SPME-GC/MS, has been described for the first time in this work. The study of the principal components showed that according to the bioactive compounds profile it is possible to differentiate between the two mustard species analysed. Moreover, the bran fraction, as a by-product of the seed processing, is a matrix rich in bioactive compounds and could be applied for the recovery of polyphenols for agro-industrial or pharmaceutical application.

1. Introduction

The term mustard refers to a group of plants of the Cruciferae family, which belongs to the genus *Brassica*. Mainly three species of mustard are cultivated worldwide for their gastronomic value: yellow or white mustard (*Sinapis alba*), oriental mustard (*Brassica juncea*), and black mustard (*Brassica nigra*). Usually, mustard plants are consumed as edible oils, condiments, sauces, fermented vegetables, or salad greens (Rahman, Khatun, Liu, & Barkla, 2018). These plants have been reported for their high nutritional value and richness in bioactive compounds such as glucosinolates, polyphenols, dietary fiber, β-carotene, and ascorbic acid (Campbell, Han, Triggs, Fraser, & Ferguson, 2012; Frazie, Kim, & Ku, 2017; Tian & Deng, 2020).

Glucosinolates are amino-acid derived compounds produced in the secondary metabolism of the Brassicaceae genus. The hydrolysis of glucosinolates by the myrosinase enzyme (thioglucoside glucohydrolase EC 3.2.3.2) produces a relevant number of biologically active compounds, such as isothiocyanates, thiocyanates, nitriles, and epithionitriles (Hanschen, Kühn, Nickel, Rohn, & Dekker, 2018). These substances exert an essential effect on plant defense, and have antimicrobial, antioomycet, antihelmintic, antimutagenic and anticarcinogenic effect (Adegbeye et al., 2020; Kamal et al., 2022; Moccellin, dos

Abbreviations: AITC, Allyl Isothiocyanate; DPPH, 2,2-Diphenyl-1-picrylhydrazyl; GAE, Gallic Acid Equivalent; GC-MS, Gas-chromatography-mass spectrometry; HS-SPME, Head-space solid phase microextraction; LRI, Linear Retention Index; OMB, Oriental Mustard Bran; OMF, Oriental Mustard Flour; PC, Principal Component; PCA, Principal Component Analysis; TPC, Total Phenolic Content; TE, Trolox Equivalent; UHPLC-ESI-MS, Ultra-high performance liquid chromatography-electrospray ionization-mass spectrometry; YMB, Yellow Mustard Bran; YMF, Yellow Mustard Flour.

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Santos, Heck, Malagi, & Dallemole-Giaretta, 2017; Poveda, Eugui, & Velasco, 2020; Traka & Mithen, 2009). Moreover, few glucosinolates as isothiocyanates are volatile and responsible for the characteristic pungent smell of Brassica plants (Bell, Oloyede, Lignou, Wagstaff, & Methven, 2018). Nowadays, allyl isothiocyanate (AITC) is one of the most studied isothiocyanates due to its antimicrobial potential and possible applications as a preservative and is derived from the hydrolysis of the glucosinolate sinigrin (Araújo, Gumiela, Bordin, Luciano, & Macedo, 2018; Bahmid, Pepping, Dekker, Fogliano, & Heising, 2020; Olaimat, Al-Holy, Abu Ghoush, Al-Nabulsi, & Holley, 2018).

In addition to glucosinolates, mustard plants are a dietary source of polyphenolic compounds with the potential to scavenge reactive oxygen species due to their electron-donating properties. In detail, mustard contain flavonoids (especially flavonols), hydroxybenzoic acids and hydroxycinnamic acids (Cartea, Francisco, Soengas, & Velasco, 2010). Among phenolic compounds, sinapic acid and sinapate esters are described as the main phenolic compounds found in the Brassicaceae family (Nguyen, Stewart, Ioannou, & Allais, 2021). Some biological properties have been associated with these metabolites such as antioxidant, antibacterial, anti-inflammatory, and UV-filter activities (Calabriso et al., 2020; Hussain et al., 2019; Mouterde, Peru, Mention, Brunissen, & Allais, 2020; Nićiforović & Abramovič, 2014).

During mustard seed processing, different products can be obtained. The mustard flour is produced by grinding the mustard seed, and the powder is used whether as an emulsifier or flavoring ingredient in culinary preparations. During the grinding, the bran fraction is discarded, and it is estimated that 60% of the mustard seed constitutes a waste product (Sehwag & Das, 2015). This by-product can be revalorized and used to recover bioactive compounds with biological properties. Some authors have addressed this issue; for instance, Reungoat et al. (2021) recovered sinapic acid from oriental mustard bran. On another approach, the bran fraction of yellow mustard has been revalued as an ingredient with antifungal properties in bread baking (Torrijos, Nazareth, Quiles, Mañes, & Meca, 2021).

To our knowledge, the phytochemical profile of the by-product bran fraction of mustard species has not been reported so far, and only a few reports were focused on yellow mustard seed bioactive compounds. Thus, to revalue this by-product and find new potential sources of bioactive compounds, the study aimed to comprehensively characterize the phytochemical profile of yellow mustard (*Sinapis alba*) and oriental mustard (*Brassica juncea*) seed fractions (flour and bran). For this, the volatile fraction was investigated using head space solid-phase microextraction (HS-SPME/CG-MS) approach, while the free and bound bioactive compounds were extracted and analysed using the UHPLC-ESI-MS/MS technique. Moreover, the total phenolic content (TPC), and antioxidant properties were investigated for all the mustard samples.

2. Material and methods

2.1. Chemicals and reagents

HPLC-grade water, acetonitrile, n-hexane and methanol (MetOH) and ethyl acetate for analysis were purchased from VWR Chemicals (Milan, Italy). Diethyl ether stabilised with BHT for analysis was obtained from PanReac AppliChem (ITW Reagents). Formic acid (>99% purity) and Folin-Ciocalteu's reagent were purchased from VWR Chemicals. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was obtained from Sigma-Aldrich (St. Louis, MO, USA). Bidistilled water was obtained with a Milli-Q system (Millipore, Bedford, MA, USA).

Chemical standards such as *p*-coumaric acid, caffeic acid, sinapic acid (98% titration), ferulic acid, *p*-hydroxybenxoic acid, rutin hydrate and gallic acid monohydrate (purity >98%) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Pure Yellow Mustard Flour (YMF) (Product code: 106), Pure Oriental Mustard Flour (OMF) (Product code: 107), Fine Yellow Mustard Bran (YMB) (Product code: 412), and Oriental Mustard Bran (Product code: 403) were obtained from G.S.Dunn (Hamilton, Canada).

2.2. Characterisation of mustard sample volatile fraction by headspace solid phase microextraction coupled with gas chromatography-mass spectrometry technique (HS-SPME/CG-MS)

For the analysis of the volatile profile of the mustard samples, the headspace solid-phase microextraction (HS-SPME), coupled with gas chromatography mass spectrometry technique (GC-MS) was employed following the methodology described by Shen et al. (2018) with minor modifications.

1 g of each mustard was placed on 20 mL glass vials, and 10 μL of an aqueous toluene standard solution (350 μg/mL) was added to each vial as reference. Headspace analyses were performed with an SPME fiber coated with 50/30 of divinylbenzene-carboxen-polidimethylsiloxane (DVB/carboxen/PDMS; Supelco, Bellefonte, PA, USA). The equilibration of the mixture was programmed for 15 min at 60 °C, while the extraction of volatile components for 50 min at 60 °C.

For GC-MS analysis, a Thermo Scientific Trace 1300 gas chromatograph coupled to a Thermo Scientific ISQ single quadrupole mass spectrometer equipped with an electronic impact (EI) source was employed (Thermo Fisher Scientific, Waltham, MA, USA). The desorption was performed at 250 °C for 3 min. The column used for chromatographic separation was a ZB-5MS capillary column (30m × 0.25 mm, 0.25 μm film thickness) (Phenomenex, Inc, Torrance, CA, USA). The temperature ramp was set as follows: 5 min at 60 °C; then raised to 180 °C at 5 °C/min and held 1 min; then raised up to 260 °C at 10 °C/min and held isothermally for 5 min. The injection was performed in splitless mode. The carrier gas used was helium at a flow of 1.4 mL/min. The detection was realised in Full Scan Acquisition Mode in a range of 40–350 m/z.

The chromatographic peaks were identified by the comparison of their registered mass spectra with those registered in NIST 14 library. Moreover, Linear Retention Indices (LRIs) were calculated based on the retention time of a C8–C20 alkane solution ran in the same conditions as the samples and then compared with the literature. A semi-quantification of the identified compounds was realised through the comparison with the internal standard (toluene). The experiments were conducted in duplicate.

2.3. Extraction of bioactive compounds from mustard samples

The extraction of the polyphenolic compounds was realised according to Martinović, Polak, Ulrih, and Abramovič (2020) with minor modifications. First, defatted samples were prepared by combining 1 g of powder with 10 mL of hexane (1:10 (w/v)) and mixing for 8 h with a reciprocating shaker at 140 strokes/min. Then, the hexane was removed by filtering through Millipore SMWP filters (5.0 μm) under vacuum, and the powders were dry overnight.

The free bioactive compounds (Fraction #1) were extracted from the defatted mustard with a mixture of methanol:acetone:water 7:7:6 (v/v) in proportion 1:10 (w/v), and then shook for 6h at room temperature. The extracts were centrifuged at 3000 rpm for 5 min, and then the powder was extracted twice with the same solvent combination but assisted with ultrasounds. The fractions were collected and extracted (liquid-liquid extraction) with a mixture of diethyl ether:ethyl acetate 1:1 (v/v) three times. The recovered ethylic phases were dried using N_2. The remaining aqueous fraction was divided into two equal parts and used to extract the bound phenolics.

Alkaline hydrolysis was realised by spiking the aqueous extract to NaOH 4 mol/L 1:2 (v/v) for 4h at 25 °C under N_2 flow and constant mixing. The extract was then acidified (pH = 2), and bioactive compounds were extracted with diethyl ether:ethyl acetate 1:1 (v/v) three times. The ethylic phases were dried completely with N_2 flow. This fraction was considered the bound bioactive compounds released after alkaline hydrolysis (Fraction #2).

Acid hydrolysis of the extracts was carried out by reflux technique. For this purpose, the extract was mixed with HCl 6M at 1:1 (v/v) and hydrolyzed at 90 °C for 45 min. Then, phenolic compounds were extracted again with a mixture of diethyl ether:ethyl acetate 1:1 (v/v). The ethylic phases were dried completely under N_2, and the result obtained was considered as the bound bioactive compounds released after performing acid hydrolysis (Fraction #3).

Previous to the injection, the dried samples were resuspended in 1 mL of methanol:H_2O (0.2% FA) 1:1, and sonicated for 5 min. Then, samples were filtered (0.45 μm, nylon membrane) and stored in glass vials.

2.4. UHPLC-MS/MS analysis of bioactive compounds in mustard

The samples (Fraction #1, Fraction #2 and Fraction #3) were injected in a UHPLC Dionex Ultimate 3000 system coupled to a triple quadrupole mass spectrometer (TSQ Vantage; Thermo Fisher Scientific Inc., San Jose, CA, USA) equipped with an Electrospray source (H-ESI II). Chromatographic separation was performed using a SunShell C18 column(2.6 μm; 2.1 i.d. x 100 mm) (ChromaNik Technologies, Osaka, Japan). The mobile phases used were water (Phase A) and acetonitrile (phase B), both acidified with formic acid (0.1%). The gradient set for analysis was the following: 0 min, 5%B; 1 min, 5% B; 6 min 40%B; 7 min 90% B; 9 min 90% B; 10 min 5% B; 17 min 5% B. For Full Scan Analysis, the gradient used for chromatographic separation was the following: 0 min, 1% B; 1 min, 1%B; 10 min 80% B; 13 min, 80% B; 14 min, 1%B; 22 min, 1%B. Flow rate was set at 0.3 mL/min and injection volume was 2 μL.

The bioactive compounds were monitored in negative ionization mode, except for sinapine confirmation that was also performed in positive ionization mode. During ESI- analyses, the spray voltage was set at 3000 V, and the capillary temperature was kept at 270 °C. Vaporizer temperature was set at 200 °C, while sheath gas (N_2) was 50 units and the auxiliary gas (N_2) was 5 units. The S-Lens RF amplitude and Collision Energy (CE) values were obtained employing an automatic function of Xcalibur software (Thermo Fisher Scientific Inc., San Jose, CA, USA). Phenolic acids were monitored using the Selected Reaction Monitoring (SRM) mode. The fragment ions monitored for *p*-coumaric acid, caffeic acid, ferulic acid, sinapic acid and *p*-hydroxybenzoic acid were the same described by Righetti et al. (2019).

In addition, analysis of unknow metabolites were carried out using full scan, scanning from m/z 100 to 1500, followed by a target MS/MS analysis with a collision-induced dissociation (CID) equal to 30 V. Pure argon gas was used for CID. The identification was performed based on the retention times (RTs), mass spectrometry data, and previous published information (Engels, Schieber, & Gänzle, 2012; Qu et al., 2020; Sun et al., 2013). Quantification of bioactive compounds was performed with calibration curves of standards (0.1–20 μg/mL) or by using the most structurally similar compound, as reported in Table 3.

2.5. Determination of the total phenolic content (TPC) and antioxidant activity

The Total Phenolic Content (TPC) and the antioxidant activity were evaluated in the mustard powders, extracting 1 g of mustard with 10 mL of MetOH:H_2O (70:30 v/v) and shook for 30 min in a reciprocating shaker at 120 strokes/min (Martelli, Cirlini, Lazzi, Neviani, & Bernini, 2020).

The TPC was determined using Folin-Ciocalteu reagent. Firstly, 250 μL of the methanolic extract or standard (gallic acid) were combined with 1 mL of Folin-Ciocalteu diluted reagent 1:10 (v/v), and then 2 mL of Na_2CO_3 (10 g/100 mL) was added. The samples were stored at room temperature in the darkness for 30 min. Then, absorbance was measured at 760 nm in a Jasco V-530 spectrophotometer (Champaign, IL, USA). A calibration curve was prepared with gallic acid (10–100 μg/mL). Results were expressed as mg of gallic acid equivalents (GAE)/kg, and the experiment was realised three times (n = 3).

For the determination of the antioxidant activity, the DPPH methodology was used. As a reference standard, a calibration curve with Trolox was prepared (0.1–1 mmol/L). Then, 0.1 mL of sample or Trolox standard were mixed with 2.9 mL of DPPH• solution (0.05 mmol/L). Also, a blank was prepared with 0.1 mL of distilled water. The samples were incubated at 25 °C for 30 min in darkness. Then, the absorbance of the sample was measured at 517 nm. The calibration curve was realised calculating the inhibition percentage (%IP) of the Trolox standard. Results were expressed as mmol/L of Trolox Equivalent (mM TE)/g and the experiments were conducted in triplicate (n = 3).

2.6. Statistical analysis

Statistical analysis was realised using GraphPad Prism 3.0 software was used (San Diego, Ca, USA). The differences ($p < 0.05$) were detected by ANOVA test followed by a post-hokey Tukey's test.

Moreover, to classify the mustard fractions, a principal component analysis (PCA) of the quantified volatile and bioactive compounds was realised using MetaboAnalyst 5.0 software (Pang et al., 2021). The features included were log transformed and mean centered.

3. Results and discussion

3.1. Volatile profile of the yellow and oriental mustard samples

The characterisation of the volatile profile of the mustard samples was realised by HS-SPME coupled to GC-MS technique. HS-SPME is a solvent-free, inexpensive, rapid and versatile method that allows the identification of trace volatiles (Balasubramanian & Panigrahi, 2010). A total of 53 compounds belonging to different chemical classes such as isothiocyanates (1), alkanes (36), ketones (5), esters (4), alcohols (5), and miscellaneous compounds (2) were detected in the mustard samples. The full identification of the detected volatile compounds, their calculated LRIs and those reported in the literature are plotted in Table 1. As expected, differences in the volatile profile were observed between the mustard species and fractions analysed (flour or bran). Moreover, a semi-quantification of the peaks identified was performed using toluene as an internal standard and the results are presented in Table 2.

The mustard sample that presented the highest concentration of volatile compounds was the OMF, with a mean value of 66.97 ± 0.11 μg/g ($p < 0.05$) (Fig. 1). This fraction of *Brassica juncea* seed was richer in volatile compounds than YMF sample, which presented an average value of 28.59 ± 4.47 μg/g of aromatic compounds. Furthermore, the analysis highlighted that the mustard bran fractions (OMB and YMB) contained lower concentration of volatile compounds when compared to their respective flours, with mean values of 27.80 ± 0.08 and 4.94 ± 0.43 μg/g, respectively.

The main characteristic volatile detected in OMF and OMB was AITC, with a mean concentration of 38.97 ± 1.09 and 6.98 ± 1.03 μg/g, respectively. In contrast, the AITC detected in YMF and YMB was lower, with a mean value of 1.21 ± 0.62 and 0.23 ± 0.04 μg/g, respectively. This chemical compound is responsible for the sulfur and pungent odor of this spice and some beneficial properties such as antimicrobial and antifungal properties (Eib, Schneider, Hensel, & Seuβ-Baum, 2020; Nazareth et al., 2018). Compared to the YMF and YMB samples, in which the AITC represented only 4.3% and 4.7% of the total aromatic compounds, respectively, AITC in the OMF and OMB represented 58.2% and 25.1%, respectively.

The greatest variety of aromatic compounds detected in all samples were alkanes, mainly linear alkanes such as n-octane, n-nonane, n-decane, n-undecane, n-dodecane, n-tridecane and n-tetradecane, as well as multiple branched alkanes. This fraction was the most representative in the YMF and YMB samples, with a mean concentration of 25.36 ± 3.94 and 3.68 ± 0.11 μg/g, implying 88.70 and 74.45% of the total

Table 1
Identification of the volatile compounds of yellow (*Sinapis alba*) and oriental (*Brassica juncea*) mustard flour and bran, with calculated LRIs, identification method, and references.

Peak	Compound	LRI calc.	LRI lit.	Identification	Reference
Isothiocyanate					
1	Allyl Isothiocyanate	880	887	MS + LRI	Engel, Baty, Le Corre, Souchon, and Martin (2002)
Alkane					
2	n-Octane	802	800	MS + LRI	Adams (2007)
3	2,4-Dimethylheptane	818	822	MS + LRI	Linstrom and Mallard (2021)
4	4-Methyloctane	856	858	MS + LRI	Linstrom and Mallard (2021)
5	n-Nonane	884	899	MS + LRI	Adams (2007)
6	2-Methylnonane	964	962	MS + LRI	Linstrom and Mallard (2021)
7	n-Decane	1000	1000	MS + LRI	Adams (2007)
8	2,5-Dimethylnonane	1013		MS	
9	2,5-Dimethylnonane like			MS	
10	2,6-Dimethylnonane	1022	1022	MS + LRI	Linstrom and Mallard (2021)
11	5-Methyldecane	1057	1056	MS + LRI	Linstrom and Mallard (2021)
12	4-Methyldecane	1061	1059	MS + LRI	Linstrom and Mallard (2021)
13	n-Undecane	1100	1099	MS + LRI	Adams (2007)
14	n-Undecane like			MS	
15	3,7-Dimethyldecane	1113	1127	MS + LRI	Linstrom and Mallard (2021)
16	5-Methylundecane	1156	1154	MS + LRI	Zaikin and Borisov (2002)
17	4-Methylundecane	1160	1158	MS + LRI	Zaikin and Borisov (2002)
18	2-Methylundecane	1165	1165	MS + LRI	Linstrom and Mallard (2021)
19	3-Methylundecane	1171	1169	MS + LRI	Linstrom and Mallard (2021)
20	2,5-Dimethylundecane			MS	
21	n-Dodecane	1200	1200	MS + LRI	Adams (2007)
22	2,4-Dimethylundecane	1209	1213	MS + LRI	Liu, Xu, and Zhou (2007)
23	2,6-Dimethylundecane	1213	1213	MS + LRI	Zeng et al. (2007)
24	4,8-Dimethylundecane	1222		MS	
25	6-Methyldodecane	1253	1253	MS + LRI	Rembold, Wallner, Nitz, Kollmannsberger, and Drawert (1989)
26	4-Methyldodecane	1259	1259	MS + LRI	Linstrom and Mallard (2021)
27	2,6,11-Trimethyldodecane	1274	1275	MS + LRI	Linstrom and Mallard (2021)
28	2,6,11-Timethyldodecane like			MS	
29	2,6,11-Trimethyldodecane like			MS	
30	2,6,11-Trimethyldodecane like			MS	
31	n-Tridecane	1300	1300	MS + LRI	Adams (2007)
32	4,6-Dimethyldodecane	1321	1325	MS + LRI	Liu et al. (2007)
33	4,6-Dimethyldodecane like			MS	
34	2-Methyltridecane	1365	1365	MS + LRI	Zaikin and Borisov (2002)
35	3-Methyltridecane	1370	1371	MS + LRI	Linstrom and Mallard (2021)
36	n-Tetradecane	1399	1399	MS + LRI	Adams (2007)
37	2,6,10-Trimetrhyltridecane	1460	1463	MS + LRI	Flamini et al. (2002)
Ketone					
38	6-Methyl-5-hepten-2-one	984	985	MS + LRI	Pino, Marbot, Payo, Chao, and Herrera (2011)
39	3-octen-2-one	1039	1040	MS + LRI	Fan and Qian (2006)
40	2(3H)-Furanone, 5-ethyldihydro	1051	1047	MS + LRI	Mahajan, Goddik, and Qian (2004)
41	3,5-octadien-2-one (E,E)	1070	1072	MS + LRI	Beaulieu and Grimm (2001)
42	3,5-octadien-2-one (E,E) like			MS	
Ester					
43	Tetradecanoic acid, 1- methylethyl ester	1823	1827	MS + LRI	Skaltsa, Mavrommati, and Constantinidis (2001)
44	Palmitic acid, methyl ester	1924	1921	MS + LRI	Santos et al. (2004)
45	9-Octadecenoic acid (Z), methyl ester			MS	
46	Octadecenoic acid, methyl ester			MS	
Alcohol					
47	1-Hexanol	864	865	MS + LRI	In et al. (2007)
48	1-Heptanol	971	970	MS + LRI	Flamini, Cioni, and Morelli (2003)
49	6-Methyl-5-hepten-2-ol	994	993	MS + LRI	Shafi, Nambiar, Clery, Sarma, and Veena (2011)
50	1-Nonanol	1173	1172	MS + LRI	Methven, Tsoukka, Oruna-Concha, Parker, and Mottram (2007)
51	1-Nonanol like			MS	
Other					
52	D-Limonene	1029	1028	MS + LRI	Angioni, Barra, Coroneo, Dessi, and Cabras (2006)
53	5-Ethylthiazole	960	959	MS + LRI	Parker, Hassell, Mottram, and Guy (2000)

aromatic compounds detected, respectively. Although a total of 20.05 ± 0.84 and 17.23 ± 1.08 μg/g of alkanes were detected in the OMF and OMB samples, they represented approximately the 30 and 62% of the total aromatic fraction, respectively. Some linear alkanes reported in the study have been identified previously through HS-SPME coupled to GC-MS technique. For instance, Wei et al. (2021) reported the presence of n-decane, n-undecane and n-dodecane in *Brassica oleracea* L. var. *capitata* L. The main difference evidenced in the methodology used by the authors with respect to our study is the previous mixture of the plant material with water and NaCl, so the volatile profile can change considerably since other volatile compounds (such as isothiocyanates and nitriles) can be generated through the myrosinase action in the

Table 2
Concentration of the volatile compounds expressed in μg/g. The samples analysed were the following: YMF: Yellow Mustard Flour; YMB: Yellow Mustard Bran; OMF: Oriental Mustard Flour; and OMB: Oriental Mustard Bran.

Compound	YMF	YMB	OMF	OMB
Isothiocyanate				
Allyl Isothiocyanate	1.21 ± 0.62^{a}	0.23 ± 0.04^{b}	38.97 ± 1.09^{c}	6.98 ± 1.03^{d}
Alkane				
n-Octane	0.37 ± 0.10^{a}	0.18 ± 0.03^{b}	n.d	1.16 ± 0.12^{c}
2,4-Dimethylheptane	0.81 ± 0.17^{a}	n.d	n.d	0.42 ± 0.03^{b}
4-Methyloctane	1.04 ± 0.07^{a}	0.19 ± 0.00^{b}	n.d	1.52 ± 0.12^{c}
n-Nonane	0.21 ± 0.04^{a}	0.13 ± 0.04^{b}	n.d	n.d
2-Methylnonane	0.16 ± 0.06^{a}	0.06 ± 0.00^{b}	n.d	n.d
n-Decane	2.10 ± 1.41^{a}	0.23 ± 0.01^{b}	0.13 ± 0.01^{b}	1.29 ± 0.04^{c}
2,5-Dimethylnonane	0.25 ± 0.02^{a}	0.07 ± 0.00^{b}	0.36 ± 0.06^{c}	n.d
2,5-Dimethylnonane like	0.14 ± 0.03^{a}	0.11 ± 0.00^{a}	0.22 ± 0.04^{b}	n.d
2,6-Dimethylnonane	0.62 ± 0.23^{a}	0.15 ± 0.04^{b}	0.83 ± 0.10^{a}	n.d
5-Methyldecane	1.48 ± 0.61^{a}	0.06 ± 0.00^{b}	1.19 ± 0.17^{a}	0.84 ± 0.09^{ab}
4-Methyldecane	1.05 ± 0.07^{a}	0.27 ± 0.02^{b}	0.95 ± 0.04^{a}	0.50 ± 0.01^{c}
n-Undecane	2.30 ± 0.12^{a}	0.54 ± 0.01^{b}	1.58 ± 0.01^{c}	3.45 ± 0.23^{d}
n-Undecane like	0.84 ± 0.11^{a}	0.20 ± 0.01^{b}	0.51 ± 0.00^{c}	0.82 ± 0.04^{a}
3,7-Dimethyldecane	0.08 ± 0.01	n.d	n.d	n.d
5-Methylundecane	0.12 ± 0.02^{a}	0.06 ± 0.00^{b}	0.08 ± 0.00^{b}	0.17 ± 0.01^{c}
4-Methylundecane	0.29 ± 0.02^{a}	0.04 ± 0.00^{b}	0.38 ± 0.01^{c}	n.d
2-Methylundecane	0.75 ± 0.03^{a}	0.14 ± 0.00^{b}	0.98 ± 0.07^{c}	0.07 ± 0.01^{b}
3-Methylundecane	0.10 ± 0.01^{a}	n.d	0.04 ± 0.02^{b}	0.29 ± 0.03^{c}
2,5-Dimethylundecane	0.42 ± 0.03^{a}	0.10 ± 0.00^{b}	n.d	n.d
n-Dodecane	3.66 ± 1.52^{ac}	0.31 ± 0.00^{b}	1.85 ± 0.11^{ab}	5.15 ± 0.15^{c}
2,4-Dimethylundecane	0.27 ± 0.05^{a}	0.04 ± 0.00^{b}	0.48 ± 0.07^{c}	n.d
2,6-Dimethylundecane	0.77 ± 0.03^{a}	0.13 ± 0.00^{b}	1.30 ± 0.07^{c}	n.d
4,8-Dimethylundecane	0.81 ± 0.06^{a}	0.10 ± 0.01^{b}	1.49 ± 0.18^{c}	n.d
6-Methyldodecane	0.72 ± 0.02^{a}	0.10 ± 0.01^{b}	1.25 ± 0.02^{c}	n.d
4-Methyldodecane	0.21 ± 0.02^{a}	0.03 ± 0.00^{b}	n.d	n.d
2,6,11-Trimethyldodecane	2.31 ± 0.10^{a}	0.23 ± 0.00^{b}	3.85 ± 0.07^{c}	0.40 ± 0.04^{d}
2,6,11-Trimethyldodecane like	0.34 ± 0.03	n.d	n.d	n.d
2,6,11-Trimethyldodecane like	0.23 ± 0.01	n.d	n.d	n.d
2,6,11-Trimethyldodecane like	0.26 ± 0.02	n.d	n.d	n.d
n-Tridecane	0.27 ± 0.01^{a}	0.02 ± 0.00^{b}	0.26 ± 0.06^{a}	n.d
4,6-Dimethyldodecane	1.23 ± 0.02^{a}	0.14 ± 0.00^{b}	1.28 ± 0.01^{a}	0.44 ± 0.04^{c}
4,6-Dimethyldodecane like	0.44 ± 0.01^{a}	n.d	n.d	0.05 ± 0.00^{b}
2-Methyltridecane	0.18 ± 0.02^{a}	n.d	0.32 ± 0.05^{b}	0.10 ± 0.01^{c}
3-Methyltridecane	n.d	n.d	n.d	0.13 ± 0.00
n-Tetradecane	0.48 ± 0.16^{a}	0.03 ± 0.00^{b}	0.58 ± 0.00^{a}	0.45 ± 0.01^{a}
2,6,10-Trimethyltridecane	n.d	n.d	0.12 ± 0.00	n.d
Ketone				
6-Methyl-5-hepten-2-one	n.d	n.d	n.d	0.37 ± 0.02
3-octen-2-one	n.d	n.d	0.24 ± 0.01	n.d
2(3H)-Furanone, 5-ethyldihydro	n.d	n.d	0.46 ± 0.00^{a}	0.11 ± 0.01^{b}
3,5-octadien-2-one (E,E)	n.d	n.d	0.53 ± 0.01^{a}	0.45 ± 0.05^{b}
3,5-octadien-2-one (E,E) like	n.d	n.d	0.21 ± 0.06^{a}	0.44 ± 0.05^{b}
Ester				
Tetradecanoic acid, 1-methylethyl ester	n.d	n.d	n.d	0.13 ± 0.02
Palmitic acid, methyl ester	0.16 ± 0.02^{a}	0.23 ± 0.10^{a}	0.23 ± 0.04^{a}	0.17 ± 0.04^{a}
9-Octadecenoic acid (Z), methyl ester	0.17 ± 0.01^{a}	0.33 ± 0.022^{a}	0.25 ± 0.02^{a}	0.13 ± 0.05^{a}
Octadecenoic acid, methyl ester	0.13 ± 0.01^{ab}	0.22 ± 0.10^{b}	0.17 ± 0.03^{ab}	0.13 ± 0.05^{a}
Alcohol				
1-Hexanol	n.d	n.d	4.98 ± 0.05	n.d
1-Heptanol	n.d	n.d	0.31 ± 0.03	n.d
6-Methyl-5-hepten-2-ol	0.14 ± 0.01^{a}	0.09 ± 0.00^{a}	n.d	1.18 ± 0.04^{b}
1-Nonanol	n.d	n.d	0.30 ± 0.03	n.d
1-Nonanol like	n.d	n.d	0.09 ± 0.01	n.d
Other				
D-Limonene	1.35 ± 0.23^{a}	0.56 ± 0.02^{b}	n.d	n.d
5-Ethylthiazole	0.07 ± 0.01^{a}	0.02 ± 0.00^{a}	0.19 ± 0.01^{b}	n.d

n.d. = not detected. Different letters mean statistical differences in the volatile content of each mustard ($p < 0.05$).

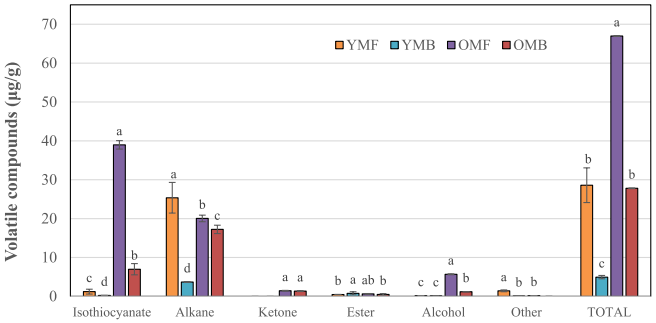

Fig. 1. Volatile composition of mustard samples. The samples analysed were the following: Yellow Mustard Flour (YMF), Yellow Mustard Bran (YMB), Oriental Mustad flour (OMF) and Oriental Mustard Bran (OMB). The means with different letters are statistically different ($p < 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

presence of water (Hanschen et al., 2018). Regarding branched alkanes in mustard, Sharma, Kumar, Kanwar, Thukral, and Bhardwaj (2017) reported farnesane and 2-methyldecane in 60 days old leaves of *Brassica juncea* after extraction with petroleum ether. Thus, our study highlighted and identified for the first time through HS-SPME technique, new linear and branched alkanes that contribute to the volatile profile of mustard seeds.

Other volatile compounds detected in smaller proportions were ketones, alcohols, and esters. Only ketones were detected in the OMF and OMB samples (1.44 ± 0.09 and 1.35 ± 0.11 μg/g, which represents 2.15 and 4.97% of total aromatic compounds, respectively). Furthermore, except for 6-methyl-5-hepten-2-ol, which was detected in the YMF, YMB and OMB, the remaining identified alcohols (1-hexanol, 1-heptanol and 1-nonanol) were found in the OMF, with an average of 5.68 ± 0.12 μg/g (implying 8.47% of total aromatic compounds). Previously, some ketones, such as 6-methyl-5-hepten-2-one and 3,5-octadiene-2-one, as well as some alcohols (1-hexanol and 1-hepatanol) have been reported in pickled and dried oriental mustard (*Brassica juncea*, Coss.) (Shen et al., 2018; Zhao, Tang, & Ding, 2007). Regarding esters, some of the compounds derived from fatty acids and determined in our samples have been reported by Sharma, Rai, and Prasad (2018) in seeds and leaves of oriental mustard (*Brassica juncea*).

Among the compounds classified as miscellaneous, a terpene, D-limonene, was identified only in the YMF and YMB samples at a concentration of 1.35 ± 0.23 and 0.56 ± 0.02 μg/g, respectively. This compound has been described in yellow mustard essential oils (*Sinapis alba*), although in traces amounts (Miyazawa & Kawata, 2006).

3.2. Bioactive compounds profile of mustard samples

Bioactive compounds in the mustard samples were analysed using UHPLC coupled to MS/MS detection. A total of 26 different bioactive compounds, including phenolic acids, flavonoids and glucosinolates, were identified in the mustard samples (Table 3). However, the distribution of these compounds changed between the mustard species and the extracted fraction analysed. Among these, 14 bioactive compounds were semi-quantified in the extracts and the results obtained are detailed in Table 4.

Phenolic acids in plants may exist in free, soluble-conjugated, and insoluble-bound forms. The conjugated forms are mainly linked to oligosaccharides and can be recovered after performing alkaline or acidic hydrolysis of the plant matrix (Gao, Ma, Wang, & Feng, 2017; Kumar & Goel, 2019). So far, most studies only focused on the free fraction, while our study characterised both free and bound phenolic compounds in the mustard powders. Thus, the remaining extract obtained after performing the extraction of the free compounds was subjected to alkaline and acid hydrolysis. It was noted that the fraction analysed after performing the akaline hydrolysis of the extracts (Fraction #2) had a lower quantity of phenolic acids in comparison with the free fraction (Fraction #1), except for sinapic acid. In all the samples, the phenolic acid content increased after performing the acid hydrolysis of the extracts (Fraction #3). These findings showed that acid hydrolysis is the best condition to release phenolic compounds and confirmed that these molecules in mustard are mainly esterified in the plant cell walls.

Regarding hydroxybenzoic acid derivatives, the yellow mustard (*Sinapis alba*) samples showed a higher content of *p*-hydroxybenzoic acid compared to oriental mustard (*Brassica juncea*). The overall content (sum of the three fractions analysed) of this phenolic acid in the YMF and YMB samples was 275.52 ± 20.82 and 226.62 ± 36.52 mg/kg, respectively, while for OMF and OMB was 34.37 ± 1.43 and 84.36 ± 17.12 mg/kg, respectively. This finding agrees with Martinović et al. (2020), who described that *p*-hydroxybenzoic acid was characteristic in *Sinapis alba* seeds. Another hydroxybenzoic acid, salicylic acid, was also identified and quantified in the mustard samples in mean values ranging from 16.12 ± 1.66 to 31.83 ± 2.68 mg/kg.

Hydroxycinnamic acid derivatives, the most common phenolic acids

Table 3
Identification of bioactive compounds from mustard samples by ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) using different MS conditions.

Compound	RT	$[M-H]^-$ (*m/z*)	MS^2 ions (*m/z*)[a]	MS mode	Std for quantification
p-Hydroxybenzoic acid	3.20	137	93	SRM	*p*-Hydroxybenxoic acid
Salicylic acid	7.32	137	93	SRM	*p*-Hydroxybenzoic acid
p-Coumaric acid	6.12	163	119	SRM	*p*-Coumaric acid
Caffeic acid	5.00	179	135	SRM	Caffeic acid
Ferulic acid	6.60	193	134, 178, 149	SRM	Ferulic acid
Sinapic acid	6.66	223	208, 164, 121	SRM	Sinapic acid
Luteolin	7.95	285	133, 151, 175, 199, 241	Full Scan	Rutin
Quercetin	7.98	301	151, 121, 107, 179, 273	Full Scan	Rutin
Kaempferol	8.59	285	185, 187, 239, 211, 255, 171, 143, 145, 151	Full Scan	Rutin
Sinapine	5.96	294 $[M-CH4]^-$ 354 $[M+HCOO]^-$ 310 $[M]+^*$	264, 236, 147, 279, 119, 164, 208 251*, 175*, 207*, 119*, 147*	Full Scan	
Hydroxybenzoic acid-*O*-hexoside	4.84	299	137, 179, 151, 113, 101	Full Scan	
Sinigrin	1.15	358	97, 96, 75, 80, 116, 195, 241, 259, 275	Full Scan	
(epi)progoitrin	1.15	388	97, 96, 75, 259, 195, 128, 136, 80, 275	Full Scan	
Sinalbin	1.88	424	97, 96,	Full Scan	

(*continued on next page*)

Table 3 (*continued*)

Compound	RT	[M-H]⁻ (*m/z*)	MS² ions (*m/z*)[a]	MS mode	Std for quantification
			75, 182, 259, 195, 80, 275, 139, 119, 231, 241		
Glucobrassicin	5.89	447	97, 96, 95, 275, 254, 191, 113, 171, 137, 80	Full Scan	
Luteolin-*O*-hexoside	7.08	447	284, 285, 255, 227, 192, 175	Full Scan	Rutin
Kaempferol-*O*-hexoside	7.24	447	284, 285, 151	Full Scan	Rutin
Hydroxyglucobrassicin	3.29	463	97, 170, 169, 255, 275, 259, 285, 239, 189, 178, 205	Full Scan	
Quercetin-*O*-hexoside	6.83	463	300, 301	Full Scan	Rutin
Sinapoyl-vanilloyl-hexoside	7.03	535	167, 205, 152, 352, 367, 123, 108, 223	Full Scan	
Disinapoyl-hexoside I	7.62	591	223, 205, 367, 352, 164, 208, 149, 190	Full Scan	
Disinapoyl-hexoside II	7.93	591	205, 223, 190, 367, 265, 247, 164, 325, 149	Full Scan	
Disinapoyl-dihexoside	7.30	753	205, 529, 223, 247, 190, 289, 208, 179, 164	Full Scan	
Trisinapoyl-dihexoside	7.62	959	735, 529, 205, 511, 223, 289, 247, 385	Full Scan	
Kaempferol-sinapoyl-trihexoside I	6.10	977	815, 609, 447, 284, 285	Full Scan	Rutin
Kaempferol-sinapoyl-trihexoside II	6.49	977	815, 653	Full Scan	Rutin

[a] Fragment ions are reported in order of relative abundance. * MS data referred to experiment in positive ionization mode.

in the plant kingdom, such as *p*-coumaric acid, caffeic acid, ferulic acid and sinapic acid, were quantified in the four mustard samples. Sinapic acid was by far the predominant component in the four mustard samples analysed, reaching a content equal to 3061 ± 338, 1981 ± 195, 2105 ± 421 and 597 ± 173 mg/kg for YMF, YMB, OMF and OMB, respectively. The ferulic acid content in YMF and YMB samples was established at 60.02 ± 4.22 and 40.29 ± 13.08 mg/kg, while for OMF and OMB samples, it was 13.15 ± 0.59 and 4.85 ± 0.68 mg/kg. Caffeic acid and *p*-coumaric acid were the minor phenolic acids, recovered at trace levels ranged from 0.67 ± 0.07 to 3.51 ± 0.98 mg/kg and 1.88 ± 0.32 to 3.73 ± 0.54 mg/kg, respectively.

Other authors have focused on phenolic compounds in different mustard plant fractions. For instance, Fang, Hu, Liu, Chen, and Ye (2008) quantified the main phenolic acids in potherb mustard (*Brassica juncea*, Coss.) and analysed the free and bound phenolic fraction after alkaline and acidic hydrolysis. The authors found that alkaline hydrolysis increased the phenolic content, contrary to our study, and reported mean values of 116 ± 1.07, 211 ± 1.09, and 15.8 ± 0.25 mg/kg for ferulic, sinapic and *p*-hydroxybenzoic acid, respectively. In our study, higher phenolic acid content was obtained after performing the acidic hydrolysis of the mustard seed fractions. Nevertheless, the values obtained previously by these authors are challenging to compare with our study because of the different extraction methodology, and moreover, the phenolic content can change considerably between plant fraction cultivars and environmental conditions (Bhandari & Kwak, 2015). In addition, ferulic acid, *p*-hydroxybenzoic acid and sinapic acid were identified but not quantified recently in yellow, oriental, and black mustard seeds by Boscariol Rasera, Hilkner, de Alencar, and de Castro (2019). Overall, the phenolic acid content was lower in the bran fractions than in the flour fraction of each mustard specie. However, it is relevant to consider that the bran is a by-product of mustard seed processing and can be used to recover these phytochemical compounds and revalue the product.

Some flavonoids, such as quercetin, luteolin, and kaempferol, were also identified in the mustard samples and quantified by comparison with a rutin standard. Luteolin was only detected in Fraction #3 of the YMB sample (0.77 ± 0.11 mg/kg). Kaempferol was exclusively found in *Brassica juncea* samples (OMB and OMF), in mean values from 1.10 ± 035 to 15.53 ± 3.66, respectively. Moreover, the ions corresponding to the loss of a hexoside (162 Da), sinapoyl-hexose (386 Da), and sinapoyl-dihexose (530 Da) allowed the identification of five glycosylated flavonoids, for instance, quercetin-*O*-hexoside (*m/z* 463), luteolin-*O*-hexoside (*m/z* 447), kaempferol-*O*-hexoside (*m/z* 447), kaempferol-sinapoyl-trihexoside I (*m/z* 977), and kaempferol-sinapoyl-trihexoside

Table 4
Quantification of the main bioactive compounds of the yellow mustard flour (YMF), yellow mustard bran (YMB), oriental mustard flour (OMF) and oriental mustard bran (OMB). Results were expressed as mean ± SD in mg/kg of mustard powder.

Bioactive compound	YMF			YMB		
	Fraction #1	Fraction #2	Fraction #3	Fraction #1	Fraction #2	Fraction #3
p-Hydroxybenzoic acid	25.02 ± 0.78^{a}	5.77 ± 1.06^{a}	244.73 ± 21.26^{a}	22.13 ± 1.90^{a}	3.05 ± 0.35^{b}	201.44 ± 35.30^{a}
Salicylic acid	7.21 ± 0.65^{a}	5.63 ± 0.35^{a}	18.99 ± 2.53^{a}	6.70 ± 0.53^{a}	3.75 ± 0.16^{b}	20.71 ± 1.15^{a}
p-Coumaric acid	0.68 ± 0.04^{a}	0.14 ± 0.03^{a}	2.45 ± 0.15^{a}	0.57 ± 0.03^{a}	0.13 ± 0.01^{a}	2.81 ± 0.95^{a}
Caffeic acid	0.18 ± 0.03^{ab}	n.d	3.55 ± 0.53^{ab}	0.12 ± 0.01^{a}	n.d	2.92 ± 1.32^{ab}
Ferulic acid	3.33 ± 0.35^{a}	1.48 ± 0.18^{ab}	60.02 ± 4.22^{a}	2.80 ± 0.29^{a}	1.26 ± 0.17^{a}	40.29 ± 13.08^{b}
Sinapic acid	74.96 ± 14.61^{a}	117.67 ± 9.10^{a}	2868.21 ± 350.51^{a}	32.08 ± 2.73^{b}	76.41 ± 17.81^{b}	1872.78 ± 118.28^{b}
Luteolin	n.d	n.d	n.d	n.d	n.d	0.77 ± 0.11
Quercetin	n.d	n.d	n.d	n.d	n.d	1.09 ± 0.12
Kaempferol	n.d	n.d	n.d	n.d	n.d	n.d
Luteolin-*O*-hexoside	0.16 ± 0.09^{a}	n.d	n.d	1.51 ± 0.25^{b}	n.d	n.d
Kaempferol-*O*-hexoside	0.66 ± 0.57^{a}	n.d	n.d	0.28 ± 0.01^{a}	n.d	n.d
Quercetin-*O*-hexoside	n.d	n.d	n.d	n.d	n.d	0.49 ± 0.05^{a}
Kaempferol-sinapoyl-trihexoside I	0.16 ± 0.10^{a}	0.18 ± 0.08^{a}	n.d	0.23 ± 0.08^{a}	n.d	n.d
Kaempferol-sinapoyl-trihexoside II	n.d	n.d	n.d	n.d	n.d	n.d
Total	112.36 ± 14.54^{a}	130.87 ± 9.27^{a}	3197.93 ± 325.29^{a}	66.42 ± 5.42^{b}	84.60 ± 18.21^{b}	2143.30 ± 232.04^{b}
Bioactive compound	**OMF**			**OMB**		
	Fraction #1	Fraction #2	Fraction #3	Fraction #1	Fraction #2	Fraction #3
p-Hydroxybenzoic acid	3.25 ± 0.31^{b}	0.40 ± 0.12^{c}	30.72 ± 1.23^{b}	2.84 ± 0.84^{b}	5.92 ± 1.19^{a}	75.60 ± 16.13^{b}
Salicylic acid	7.10 ± 1.01^{a}	2.48 ± 0.93^{bc}	21.87 ± 2.72^{a}	2.56 ± 0.45^{b}	2.15 ± 0.43^{c}	11.42 ± 1.68^{b}
p-Coumaric acid	0.32 ± 0.04^{b}	0.07 ± 0.01^{b}	0.95 ± 0.07^{b}	0.25 ± 0.07^{b}	0.05 ± 0.02^{b}	0.37 ± 0.05^{b}
Caffeic acid	0.32 ± 0.12^{b}	n.d	4.78 ± 0.32^{a}	0.14 ± 0.05^{a}	n.d	1.75 ± 0.29^{b}
Ferulic acid	0.97 ± 0.10^{b}	0.10 ± 0.03^{a}	13.15 ± 0.59^{c}	0.89 ± 0.29^{b}	5.28 ± 3.04^{b}	4.85 ± 0.68^{c}
Sinapic acid	132.60 ± 37.83^{c}	109.81 ± 5.84^{a}	1862.31 ± 445.11^{b}	8.73 ± 2.75^{b}	38.96 ± 7.86^{c}	549.33 ± 167.97^{c}
Luteolin	n.d	n.d	n.d	n.d	n.d	n.d
Quercetin	n.d	n.d	4.44 ± 0.30	n.d	n.d	n.d
Kaempferol	n.d	n.d	15.53 ± 3.66^{a}	n.d	n.d	1.10 ± 0.35^{b}
Luteolin-*O*-hexoside	0.38 ± 0.16^{ac}	3.16 ± 0.29^{a}	n.d	0.74 ± 0.18^{c}	0.60 ± 0.11^{b}	n.d
Kaempferol-*O*-hexoside	n.d	n.d	n.d	n.d	n.d	n.d
Quercetin-*O*-hexoside	n.d	n.d	18.44 ± 2.39^{b}	n.d	n.d	0.56 ± 0.05^{a}
Kaempferol-sinapoyl-trihexoside I	3.84 ± 0.46^{b}	8.81 ± 0.94^{b}	n.d	0.86 ± 0.45^{a}	0.86 ± 0.10^{a}	n.d
Kaempferol-sinapoyl-trihexoside II	0.10 ± 0.03	0.59 ± 0.37	n.d	n.d	n.d	n.d
Total	148.87 ± 38.37^{c}	125.43 ± 4.28^{a}	1972.18 ± 448.65^{b}	17.01 ± 5.04^{d}	53.82 ± 12.44^{b}	644.99 ± 185.50^{c}

Fraction #1 is referred to free bioactive compounds; Fraction #2 is referred to bioactive compounds released after alkaline hydrolysis; Fraction #3 is referred to bioactive compounds released after acid hydrolysis. Different letters mean statistical differences for each mustard variety according to the fraction analysed ($p < 0.05$). n.d. = not detected.

II (*m/z* 977). Flavonols such as quercetin and kaempferol are characteristic in *Brassica* plants and are commonly found as O-glycoside forms (Cartea et al., 2010). These compounds were detected in a range between 0.10 and 18.44 mg/kg, according to the sample analysed, and the quercetin-*O*-hexoside was the most abundant flavonoid recovered in Fraction #3 of the OMF.

Other polyphenolic compounds were tentatively identified in the mustard samples (Table A1). The ions corresponding to the loss of a hexose moiety (162 Da), sinapic acid (224 Da), sinapoyl-hexose moiety (368 Da), sinapoyl-dihexose moiety (530 Da), were indicative of six additional glycosylated phenolics (hydroxybenzoic-*O*-hexoside, sinapoyl-vanilloyl-hexoside, disinapoyl-hexoside I, disinapoyl-hexoside II, disinapoyl-dihexoside and trisinapoyl-dihexoside). These compounds were mainly identified in Fraction #1 and Fraction #2 of the mustard samples. Moreover, sinapine, a sinapic acid choline ester, was also identified in the free and bound fractions of the mustard seeds. The reported values for this compound range from 4 to 29.2 g/kg in defatted seeds, and it is estimated that represents the 90% (w/w) of the sinapic acid derivatives (Flourat, Willig, Teixeira, & Allais, 2019; Martinović et al., 2020; Mayengbam, Aachary, & Thiyam-Holländer, 2014). The bioactive profile was in accordance with Engels et al. (2012), who identified the glycosylated esters of sinapic acid in oriental mustard (*Brassica juncea* L.) seed meal. Thus, the study showed a chemical diversity in polyphenolic composition, mainly sinapic acid derivatives in both mustard seeds.

In addition to the other bioactive compounds, up to 5 glucosinolates were identified in Fraction #1 and Fraction #2 of the mustard seeds (Table A1). Sinigrin, sinalbin, progoitrin and glucobrassicin were detected in all mustard samples, while hydroxyglucobrassicin was only identified in the OMF sample. Among the glucosinolates identified, the most representative described in oriental mustard (*Brassica juncea*) is sinigrin and its main hydrolysis product by myrosinase action the AITC, an isothiocyanate that has been previously detected in the aromatic fraction analysis (Herzallah & Holley, 2012). Likewise, in yellow mustard (*Sinapis alba*), the characteristic glucosinolate described is sinalibin, that generates *p*-hydroxybenzyl isothiocyanate, a non-volatile antimicrobial compound with low stability, which could explain its absence in the previous analysis performed (Borek & Morra, 2005; Monu, David, Schmidt, & Davidson, 2014). Furthermore, the other glucosinolates identified in our mustard seeds are precursors of isothiocyanates with beneficial properties, such as 3-indolymethyl isothiocyanate (glucobrassicin), (2R)-Hydroxybut-3-enyl isothioacyanate (progoitrin) and 4-Hydroxy-3-indolylmethyl isothiocyanate (hydroxyglucobrassicin). These compounds have been reported in mustard and other cultivars of the Brassica family, such as broccoli, cauliflower and cabbage, among others (Melrose, 2019; Qu et al., 2020).

3.3. Total phenolic content and antioxidant activity of mustard samples

The study determined the TPC in the mustard samples after extraction using the Folin-Ciocalteu reagent and the results are shown in Fig. 2a. The YMF sample presented the higher total phenolics content (22997 ± 797 mg/kg of GAE) compared to the rest of samples analysed (p value < 0.05). It was noted that YMB also presented a high value of TPC in comparison to OMF and OMB (8601 ± 776, 8175 ± 98 of mg GAE/kg, respectively), with an average of 17136 ± 785 mg/kg of GA

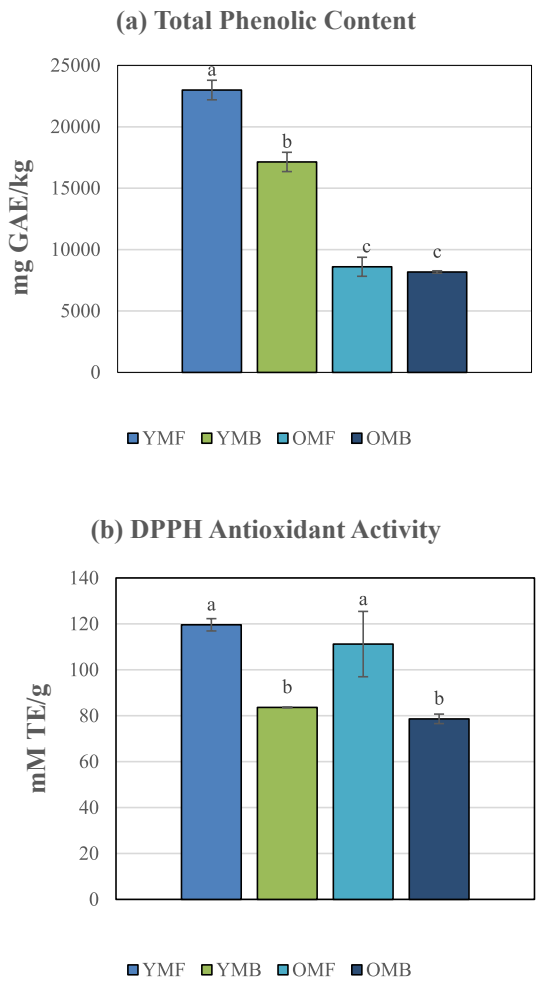

Fig. 2. Total Phenolic Content (TPC) (a) and Antioxidant activity (b) of mustard samples. The samples analysed were the following: Yellow Mustard Flour (YMF), Yellow Mustard Bran (YMB), Oriental Mustad flour (OMF) and Oriental Mustard Bran (OMB). Different letters mean statistical differences ($p < 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

equivalents. Previous studies have determined the TPC of yellow and oriental mustard seeds. However, there are no reports focused exclusively on the mustard bran fraction. Depending on the solvents employed, different TPC values have been reported for mustard seeds and plant fractions. Similar results to our study were obtained by Boscariol Rasera et al. (2019), which established a TPC content ranging from 0.82 to 20.00 mg GAE/g for *Sinapis alba* seeds, while TPC ranged from 0.65 to 12.16 mg/g for *Brassica nigra* seeds. Also, TPC had been established in fresh *Brassica juncea* leaf extracts, for instance, Subudhi and Bhoi (2014) had reported 23.1 mg GAE/100 g.

Moreover, the antioxidant activity was measured as DPPH scavenging activity and the results obtained are plotted in Fig. 2b. Both yellow and oriental mustard flours (YMF and OMF) showed higher antioxidant activity (119.6 ± 2.7 and 222.2 ± 14.2 mmol TE/g, respectively) in comparison with their bran fractions (83.6 ± 0.2 and 78.6 ± 2.0 mmol TE/g for YMB and OMB, respectively) ($p < 0.05$). These differences between flour and bran fractions could be explained by the presence of bioactive compounds that was not possible to quantify in our samples but with a strong DPPH radical scavenging activity, such as sinapine or other sinapic acid derivatives (Thiyam, Stöckmann, Zum Felde, & Schwarz, 2006).

3.4. Principal component analysis (PCA)

In order to stress the differences in the production of bioactive metabolites between the mustard fractions and species (*Sinapis alba* or *Brassica juncea*), a PCA analysis was performed with the quantification of all the compounds detected in the samples, both volatile and bioactive compounds (phenolic acids and flavonoids), and the results obtained are shown in Fig. 3. The sum of the first two principal components (PC) reached 75.6% of the total variance, of which PC1 represented 41.4% and PC2 34.2% of the total variance. The samples analysed were distinguished in four different clusters (Fig. 3a). The PC1 distributed the YMF, YMB and OMB on the negative axis, while OMF was positioned on

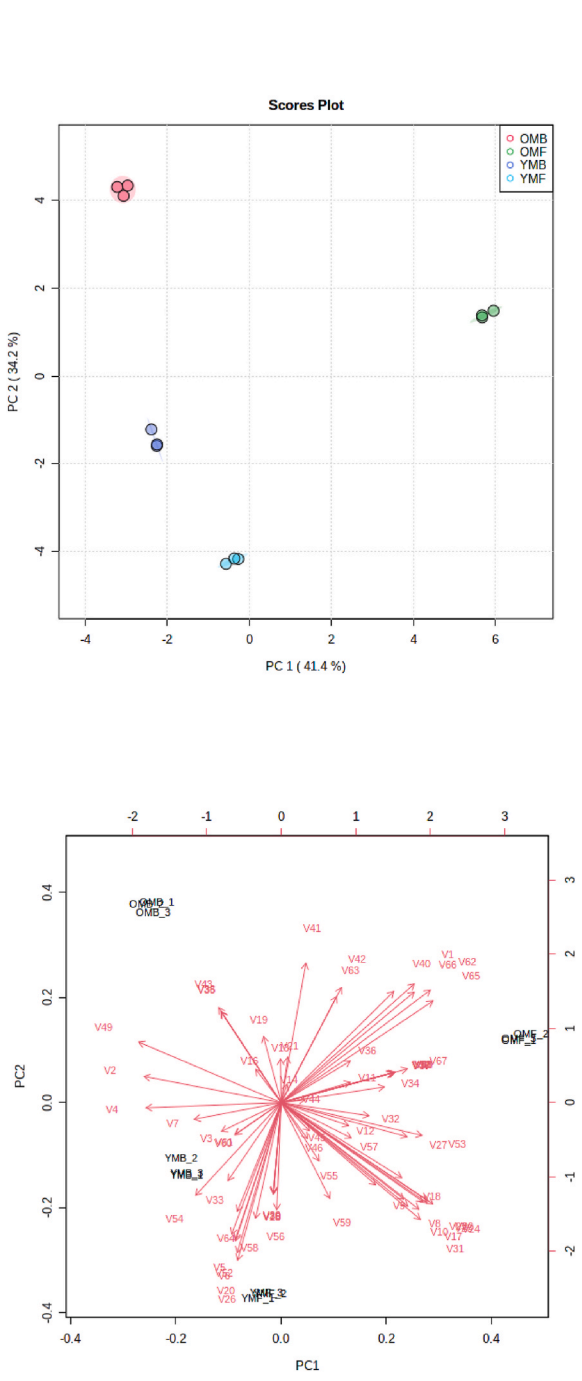

Fig. 3. Scatter plot of scores from PC1 vs. PC2, obtained using the concentrations of the volatile and the bioactive compounds found in the mustard samples (a) and relative loadings of the variables used (b).

the positive axis. The PC2 differenced the two species of plants analysed, *Sinapis alba* (on the negative axis) and *Brassica juncea* (positive axis) and confirmed the particularity of each mustard specie based on its bioactive compounds.

The loading plot is shown in Fig. 3b and presents the relative importance of the variables analysed. The YMF and YMB fraction presented a similar profile according to the first component and were distinguished between each other based on the second component. The YMB fraction were distinguished from the YMF fraction mainly for the presence of some flavonoids, such as luteolin (V60) and quercetin (V61) that were characteristic of this seed fraction, The phenolic acids that influenced the statistical differences among *Brassica juncea* seed fractions were the higher *p*-hydroxybenzoic (V54), ferulic (V58) and *p*-coumaric (V56) content quantified in the *Sinapis alba* seed fractions. Moreover, some volatile alkanes, such as 4-methyldodecane (V26), 2,5-dimethylundecane (V20), n-nonane (V5), and 2-methylnonane (V6), were characteristic of *Sinapis alba* seed fractions.

Regarding OMB, the variables that influenced the separation from the other samples were the presence of 6-methyl-5-hepten-2-ol (V49), tetradecanoic acid, 1-methyl ethyl ester (V43), and 6-methyl-5-hepten-2-one (38), which were characteristic of this seed fraction. Concerning OMF, higher AITC (V1) content and the identification of some glycosylated flavonoids allowed the differentiation from the OMB, YMF and YMB; for instance, kaempferol-sinapoyl-trihexoside I (V66), kaempferol (V62), quercetin-O-hexoside (V65) and kaempferol-sinapoyl-trihexoside II (V67). Moreover, other metabolites were only found in *Brassica juncea* seed fractions, such as 3,5-octadien-2-one (41), 2(3H)-furanone, ethyl dihydro (V40) and 2-methyltridecane (V34).

The samples analysed presented some particularities in aromatic compounds or their polyphenol profile, allowing the distinction between mustard species (*Sinapis alba* or *Brassica juncea*) and fractions. Hence, these differences should be considered for further uses of the mustard seeds, either in food or for recovering of the phytochemicals for pharmaceutical applications.

4. Conclusions

In our study, a total of fifty-three volatile compounds were identified through HS-SPME coupled to GC-MS technique, and twenty-six different phenolic compounds (including phenolic acids, flavonoids and glucosinolates) were determined in the mustard seed fractions. The bioactive profile obtained was characteristic of each mustard fraction and specie and allowed its distinction when the PCA was applied. On the one hand, yellow mustard (*Sinapis alba*) presented a higher average content of phenolic acids such as sinapic, *p*-hydrozibenzoic, and ferulic acids compared to oriental mustard (*Brassica juncea*) for both brain and flour faction. Precisely, the bran fraction, a by-product product of mustard seed processing, and proved to be a rich source of bioactive compounds. On the other hand, oriental mustard (*Brassica juncea*) demonstrated some particularities, such as a higher allyl isothiocyanate content and the presence of glycosylated flavonoids that were not found in yellow mustard (*Sinapis alba*) seed fractions.

Therefore, mustard bran could be exploited for the extraction and revalorization as a source of bioactive compounds with biological and health-promoting effects. Nevertheless, further investigations should study strategies for recovering these phytochemicals from the mustard by-products, with a special focus on the bound phenolics since they represent a relevant fraction of the bioactive components in mustard seeds.

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CRediT authorship contribution statement

Raquel Torrijos: Methodology, Formal analysis, Writing – original draft. **Laura Righetti:** Validation, Investigation, Writing – review & editing. **Martina Cirlini:** Validation, Supervision, Visualization, Writing – review & editing. **Luca Calani:** Validation, Visualization, Investigation, Writing – review & editing. **Jordi Mañes:** Project administration, Funding acquisition. **Giuseppe Meca:** Supervision, Project administration, Funding acquisition. **Chiara Dall'Asta:** Resources, Supervision, Project administration.

Declaration of competing interest

None.

Data availability

No data was used for the research described in the article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2022.114221.

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